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Antibacterial Activity Of Manganese-Doped Zinc Oxide Nanoparticles Against Multidrug Resistant Salmonella Typhi

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Abstract

Background: Multiple drug resistant (MDR) Salmonella Typhi is a public health threat. Due to their unique physicochemical properties, the manganese doped with zinc oxide nanoparticles (Mn-ZnO NPs) have become of interest as possible antimicrobial agents.
Background: Multi drug resistant (MDR) Salmonella
Objective: This research aims at synthesis and characterization of Mn-ZnO NPs and its antibacterial activity and hemocompatibility.
Methodology: Chemical co-precipitation method was used for the synthesis of Mn-ZnO NPs. UV–Vis, XRD, EDX, SEM and FTIR were employed to characterize the nanoparticles. Red blood cell (RBC) hemolysis assay was used to evaluate hemocompatibility of red blood cell (RBC). The antibacterial activity against the MDR S. Typhi was determined by agar well diffusion method and minimum inhibitory concentration (MIC) was determined by broth based assay.
Results: The absorption peak of Mn-ZnO NPs is 372 nm and the pure ZnO NPs is 360 nm in the UV-Vis spectroscopy. XRD patterns display the hexagonal wurtzite structure and EDX analysis revealed the presence of zinc (56.3%), oxygen (38.2%) and manganese (5.5%). A few agglomeration and 40-80 nm particles were identified by the SEM. FTIR spectra showed typical peaks at 3412, 1635 and 435 cm-1. Hemolysis was 4.5% at 50 µg/mL and increased to 10.1% at 100 µg/mL. The diameter of the inhibition zone grew from 8.4 mm at 20 µg/mL to 16.8 mm at 60 µg/mL concentrations. The MIC of the MDR S. Typhi was 62.5 µg/mL.
Conclusion: The concentration-dependent antibacterial activity of the Mn-ZnO NPs against MDR S. Typhi was observed and the relatively low hemolytic activity of the NPs was observed at low concentration. They need further investigation for their antimicrobial activity, action mechanism and for their safety concerning potential biomedical applications.

Introduction

The occurrence of extensively drug-resistant (XDR) S. Typhi has added to the difficulties in treatment and the need to explore novel antimicrobial strategies. [1–3]. Mechanism of their antibacterial activity may be associated with the generation of reactive oxygen species (ROS), damage to membrane and release of zinc ions depending on the particle size, concentration, morphology and experimental conditions [4–6].

Doping of metal oxides with transition metals is one of the methods to change their structural, optical and surface properties. The electronic properties of Mn-ZnO NPs can be different from those of the undoped ZnO NPs. The changes might affect their interactions with bacterial cells and their antimicrobial activity. The impact of the manganese incorporation, however, is dependent on the synthesis conditions, the concentration of the dopant and the properties of the nanoparticles. Thus, a detailed physicochemical characterisation is required to evaluate the properties of synthetic nanoparticles and their possible biological effects [5-7].

METHODOLOGY

Zinc oxide nano particles were prepared through chemical co-precipitation method. Zinc nitrate hexahydrate was dissolved in distilled water and stirred at 45°C and then, potassium hydroxide solution was added slowly drop wise to it with continuous

stirring. In the case of manganese doped ZnO nanoparticles, manganese nitrate tetrahydrate was introduced in the zinc precursor solution prior to titration. Synthesized nanoparticles were tested for their anti-bacterial activity using agar well diffusion method. The bacterial cultures were prepared and tested with the concentrations of nanoparticles (50, 100, 200 µg/mL) on Mueller–Hinton agar. Distilled water was used as negative control, and streptomycin was used as positive control. The zones of inhibition were measured after a 24 hour incubation at 37°C with Vernier calipers. The minimum inhibitory concentration (MIC) was measured by a 96-well microplate method and spectrophotometrically measured after 24 hours incubation. Experiments were repeated three times to enhance the reliability of the results.

Descriptive and inferential statistics were used to analyze the data. The results were given in the form of mean ± SD and compared between the treatment group and the control group using Student's t test. Statistically significant differences were determined using a p value < 0.05. The antibacterial activity of the synthesized nanoparticles was presented in the form of graphs, made by using the software Microsoft Excel and Origin.

RESULTS

The colour of the solution changed from milky white to pale yellow thus assisting the formation of Mn-ZnO NPs. Characterization results showed the presence of nanoscale particles with the presumed crystal structure of ZnO and with a detectable amount of Mn.

The higher the concentration of the nanoparticles, the more hemolysis there was. Hemolysis was 4.5% at 50 µg/mL indicating low hemolytic activity under the study conditions at this concentration.

The antibacterial activity of the nanoparticles was shown to be concentration-dependent. Inhibition zone is wider in higher concentrations of the antibiotic, i.e. 60 µg/mL; which shows that there is more inhibition of bacterial growth.

The MIC of Mn-ZnO NPs was calculated to be 62.5 µg/mL against MDR Salmonella Typhi, the lowest concentration tested in which bacteria were not visibly growing. In general, the results indicate potential antibacterial properties, but additional studies are needed to determine the safety and efficacy of these compounds in biological systems.

Table 1. Physicochemical Characterization of Mn-ZnO NPs

Technique	**Results**	**Interpretation**
UV–Vis spectroscopy	Peak at 372 nm (pure ZnO: 360 nm)	Red shift indicated altered optical properties
XRD	Peaks at 31.7°, 34.4°, 36.2°, 47.5°, 56.6°, and 62.9°	Consistent with hexagonal wurtzite ZnO
EDX	Zn: 56.3%; O: 38.2%; Mn: 5.5%	Confirmed the presence of manganese
SEM	Particle size approximately 40–80 nm	Spherical to slightly hexagonal particles with some agglomeration
FTIR	3412, 1635, and 435 cm^{-1}	O–H stretching, O–H bending, and Zn–O stretching, respectively

Table 2. Hemolytic Activity of Mn-ZnO NPs

Concentration (µg/mL)	**Hemolysis (%)**
10	0.9
25	2.1
50	4.5

75	7.3
100	10.1

Table 3. Antibacterial Activity Against MDR Salmonella Typhi

Concentration (µg/mL)	Zone of inhibition (mm)
20	8.4
40	12.6
60	16.8

Table 4. Minimum Inhibitory Concentration (MIC)

Concentration (µg/mL)	Bacterial growth
125	No growth
62.5	No growth
31.25	Growth
15.625	Growth

DISCUSSION

The synthesis, physicochemical properties, hemocompatibility and anti-bacterial activity of Mn-ZnO NPs against MDR S. Typhi were investigated in the present study. The results showed presence of detectable manganese and characteristic peaks of ZnO crystalline structure and antibacterial activity in a concentration dependent manner. These results indicate that there is a need to investigate further the use of Mn-ZnO NPs as antimicrobial materials.

The UV–Vis absorption peak of pure ZnO NPs was shifted from ~360 nm to 372 nm for the Mn-ZnO NPs. The red shift observed here has been attributed to changes in optical properties of ZnO due to incorporation of manganese, which may be a consequence of electronic structure change of the material. In metal-oxide nanomaterials, similar optical modifications have been studied but the exact mechanism should be further studied [4–6]. The diffraction peaks by XRD showed the hexagonal wurtzite structure of ZnO. The presence of manganese in the synthesized material was supported by the detection of zinc (56.3%), oxygen (38.2%), and manganese (5.5%) by EDX. But these are not conclusive evidence of the exact lattice position or manganese oxidation state, because the EDX spectra can overlap.

SEM images revealed that the particles had the shape of a sphere or slightly hexagonal, approximately 40 to 80 nm and there was some agglomeration. Their size, which is at the nanoscale, could allow them to interact better with the surface of the bacteria. The FTIR analysis gave the bands of 3412, 1635 and 435 cm-1 which were associated to O–H stretching, O–H bending and Zn–O stretching, respectively. The results are in line with the formation of the surface associated hydroxyl groups in the nanoparticles of ZnO. The previous studies have reported that ZnO NPs have inhibitory effects on the growth of bacteria by the production of ROS, disruption of the membrane and release of zinc ions [4-6]. Nevertheless, these mechanisms were not directly investigated in the present study.

The result has implications in the light of the emerging problem of resistance of typhoid fever in Pakistan which has been reported in XDR S. Typhi strains [1–3,8,9]. This outcome corroborates with evidence of inhibitory activity of the nanoparticles. The MIC should be confirmed, however, by standardized broth microdilution methods with the correct blank(s) for the nanoparticles, and with the proper growth and sterility control(s).

Conclusion:

The study indicates that Mn-ZnO NPs have a potential in-vitro antibacterial activity against MDR S. Typhi. However, their potential applications need to be validated by

replicated experiments, statistical comparisons, antibiotic controls, MBC determination, mechanistic studies, and thorough biocompatibility studies. These investigations will aid in determining the reproducibility, safety, and possible clinical relevance of the synthesized nanoparticles.

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